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Feverish interactions between fungi, bacteria and rocks … in the dark of a volcanic cellar

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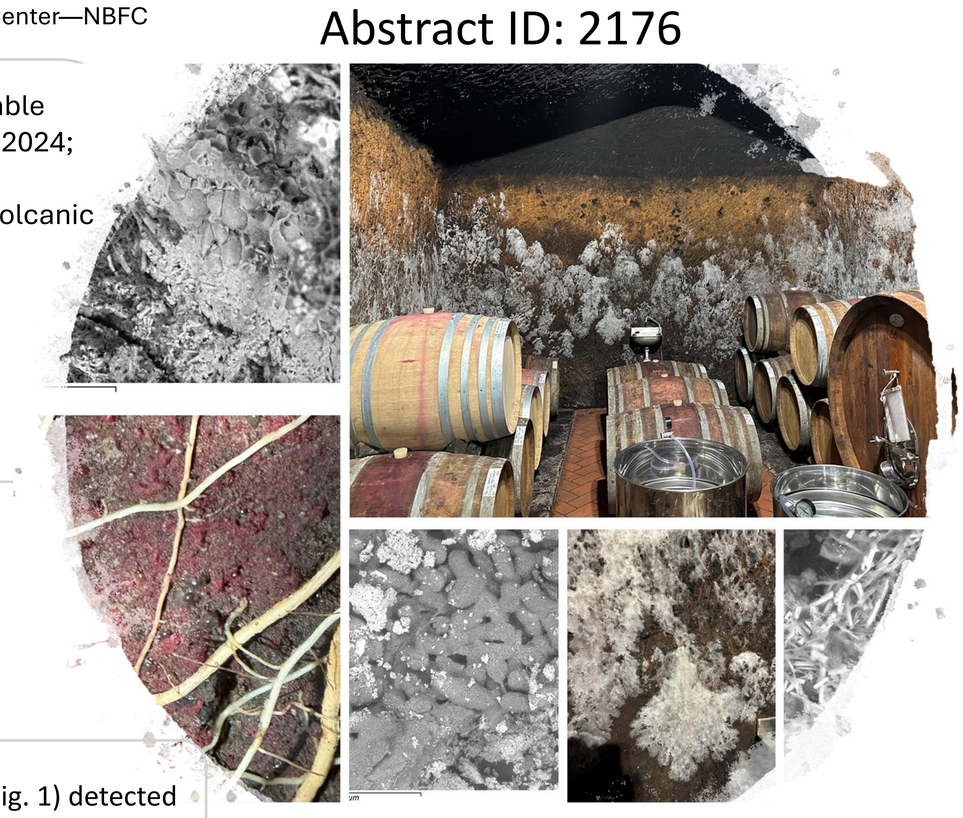
Background

Hypogean volcanic communities are unique ecological niches that support microbial communities living under stable conditions, including constant temperature, high humidity, low light, and a distinct rock composition (Turrini et al., 2024; Martin-Pozas et al., 2020). Fungi play key roles in biogeochemical processes, such as mineral weathering, oxalate secretion, and biofilm development on mineral substrates (Finlay et al., 2020). However, the fungal community in volcanic tuff caves is poorly understood, particularly in terms of its interactions with bacteria and mineral substrates.

This study focused on an underground cellar of wine barrels near Pitigliano (Central Italy), dug into red tuff with black scoria of the Sovana ignimbrite formation. It has a stable microclimatic environment and is used for wine production by Sassotondo Organic Farm. We investigated dominant fungal species through both culturing and culture-independent techniques. Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) were also employed to examine microbial-mineral interactions.



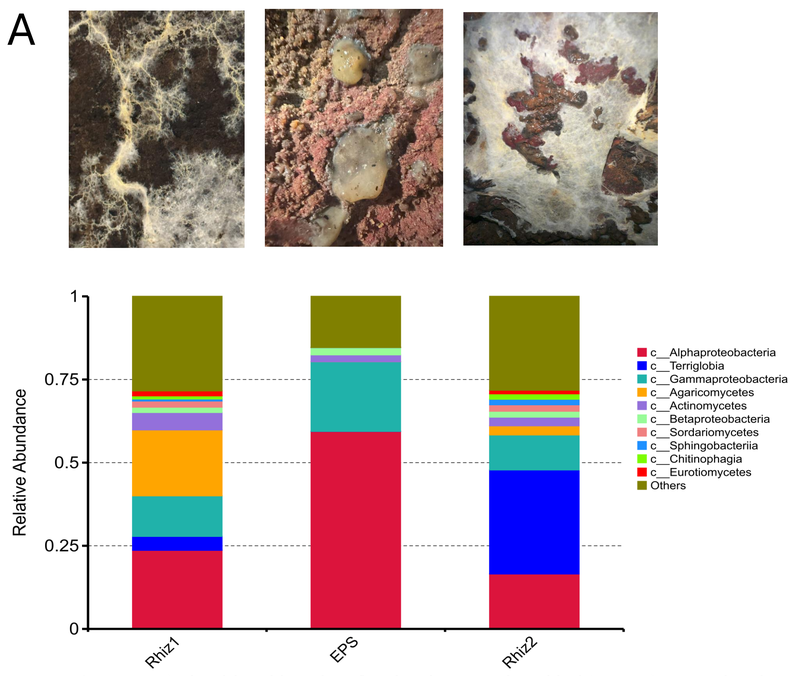


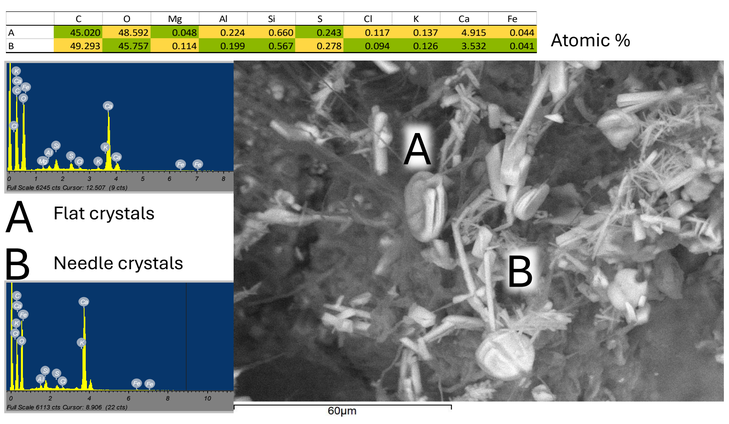
Figure 1. A) Classl-level barplots for the three analysed habitats. **B)** Genus-level heatmap with Phylum and Group (RM=Rhizomorph; EB=Extracellular Biofilm)

Metagenomics

Shotgun metagenomics (Fig. 1) detected a higher abundance of *Piloderma*, *Fibularhizoctonia*, and *Athelia* genera from the fungal rhizomorphs (with all three belonging to: Class Agaricomycetes, Order: Atheliales, Family: Atheliaceae). Several taxa of bacteria, particularly *Sphingomonas*, were observed in close association with fungal mycelia. Functional profiling by CAZy and EggNOG (Fig. 4) showed habitat-specific metabolic strategies: Rhiz2 possessed the highest lignocellulose breakdown potential (high GH content) and stress-related transport mechanisms (e.g., TonB-dependent receptors). EPS had higher levels of GT enzymes and biosynthetic, transcriptional, and transport activities (e.g., ABC transporters, ATPase activity). Despite apparent environmental homogeneity of the hypogeum, there was an evident microscale heterogeneity, with distinct fungal species ruling rather isolated patches of the walls.

SEM–EDS Analysis

SEM-EDS analysis revealed numerous crystals embedded within fungal structures, suggesting a biogenic origin (Fig.2). Calcium oxalate crystals of varying shapes were observed forming directly on rhizomorphs, likely resulting from fungal-mediated calcium translocation and the secretion of organic acids. In addition, crystals rich in Si, Al, and K, possibly zeolites, were identified on biofilm-covered tuff surfaces.



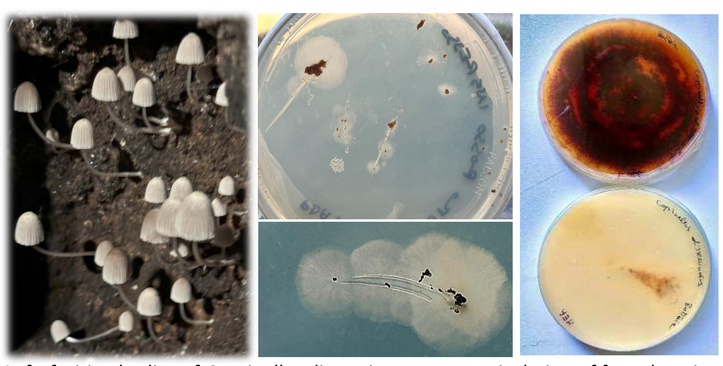


Figure 3. Left: fruiting bodies of *Coprinellus disseminatus*; centre: isolation of fungal strains from sampled rizomorphs; right, *C.disseminatus* cultures (on top an ozonium, sterile dark mycelium typical of this species)

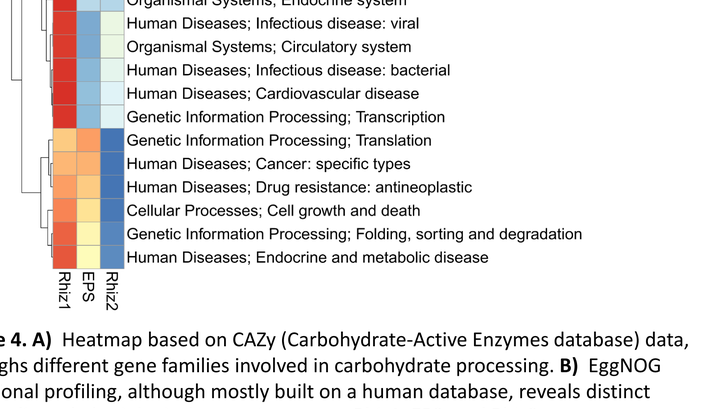


Figure 4. A) Heatmap based on CAZy (Carbohydrate-Active Enzymes database) data, highlighs different gene families involved in carbohydrate processing. **B)** EggNOG functional profiling, although mostly built on a human database, reveals distinct metabolic and physiological strategies among Rhiz1, EPS, and Rhiz2.

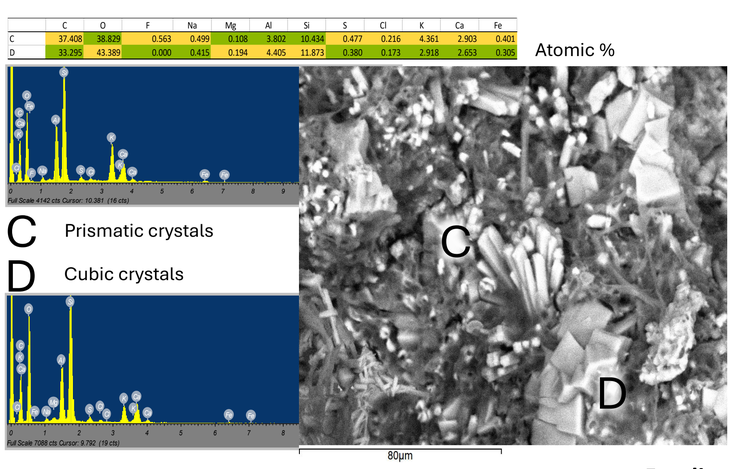


Figure 2. A) Flat oxalate crystals **B)** needle crystals of calcium oxalate. **C)** Prismatic and **D)** cubic crystals, presumably zeolites (XRD will be used to verify their nature).

Fungal Isolation and Culturing

Fruiting bodies and fragments of rhizomorphs sampled from different areas were used to obtain fungal cultures by direct inoculation on MEA and PDA media containing antibiotics, followed by further cleaning and separation of the different fungal isolates (Fig. 3). The incubation process took place at 25 °C for an extended period of 15 days. Among the strains which emerged as dominant were *Coprinellus disseminatus*, and strains of *Mortierella* sp. and *Leptosporomyces* sp. the latter from *Agaricomycetes* class, order *Atheliales*.

Take-home message

Several fungal taxa (e.g., *Piloderma*, *Fibularhizoctonia*, *Athelia, Coprinellus*) and associated bacteria (i.e. *Sphingomonas*) inhabit volcanic tuff cellar walls, exhibiting niche partitioning and specialised metabolic strategies (e.g., lignocellulose degradation, EPS production, organic acids exudation). Fungi mediate mineral transformations, as evidenced by the concentration of microelements (Mg, K, S, Na, Ca, Fe) in the mycelium and the precipitation of calcium oxalate crystals, highlighting the fungal role in tuff rock weathering.

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